Conclusions: We have generated nanoparticles that can serve as a carrier system to deliver clinically relevant disease modifying osteoarthritic drugs in a more effective way after intra-articular injection. We are currently investigating the retention of nanoparticles in the joint and are developing strategies to target these particles to cartilage.

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MEDINGEL™: A CONTROLLED-RELEASE PLATFORM FOR INTRAARTICULAR DELIVERY OF OSTEOARTHRITIS DRUGS

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Purpose: Current osteoarthritis treatments have clear delivery limitations and are principally designed to treat symptoms. Drawbacks include inefficient penetration of drugs into the joint space, systemic side effects and short duration of action. Drugs administered for systemic release have poor synovial penetration that is required to provide relief from OA-related pain and inflammation. High plasma concentrations of many NSAID compounds lead to severe side effects such as stomach pain and ulcers. And in the case of orally administered celecoxib, warnings of increased risk for heart attack and stroke have been issued. MedinCell will present our company's experience in designing polymer-based delivery technology to circumvent these problems with the goal of improving osteoarthritis treatments. We will discuss the use of our MedinGel™ platform for multi-month local delivery of small molecules and biologics to help control pain, reduce inflammation and promote cartilage repair. Without controlled release capability, drugs injected directly into the joint spaces are often cleared within hours. Yet daily intraarticular injections are untenable, as they would have to be administered by trained clinicians and would severely impact the patients' quality of life. Additionally, a high frequency of injections into the joint could have increased risk of bacterial infection. By tailoring formulations to specific drugs for a range of delivery durations, MedinCell is confident that intraarticular formulations can deliver a sustained therapeutic drug concentration over multiple months to obviate current concerns and improve both treatment efficacy and patient compliance.
Methods: After evaluation of drug solubility and set up of in vitro-release assays, a series of sets of formulations evaluating combinations of polymers at different concentrations were prepared. The MedinGel matrix is composed of mixtures of drug molecules with solubilized, bioresorbable polymers. Within minutes after injection through a fine needle, the aqueous environment of the joint leads to a phase-separation of the formulated polymers and formation of a three-dimensional matrix. This resulting semi-solid hydrogel depot entraps and protects drug molecules within the joint space, and minimizes clearance to the plasma.
Results: Candidate formulations that provided a range of drug release rates in vivo were evaluated. The best candidates were then tested by intraarticular injection. MedinCell has designed intraarticular formulations of OA drug therapies that maintained therapeutic synovial concentrations for a 3-month duration in animals.
Conclusions: The MedinGel platform enables control over the initial drug burst characteristics, allowing the remainder of the drug to be released over a predetermined duration via polymer hydrolysis and drug diffusion until the depot is completely resorbed. No covalent modification of the active substance is required, which can simplify regulatory and development pathways. We will present data showing differential compartmentalization of drug between joint and plasma when comparing normal injection to MedinGel delivery. Additionally, MedinCell expects that this technology will provide effective delivery of disease-modifying caspase inhibitors and proteins (such as BMP7) for trauma-induced arthritis.

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THE CHONDROPROTECTIVE ROLE OF CITED2 IN POST-TRAUMATIC OSTEOARTHRITIS

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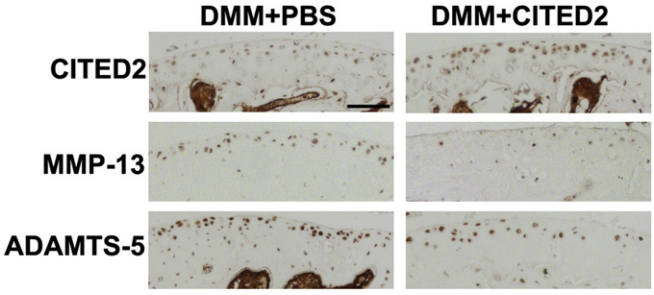
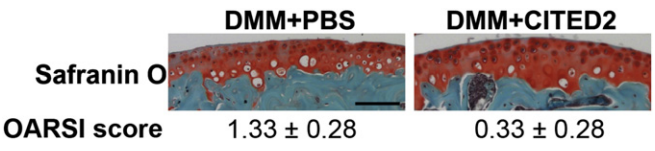
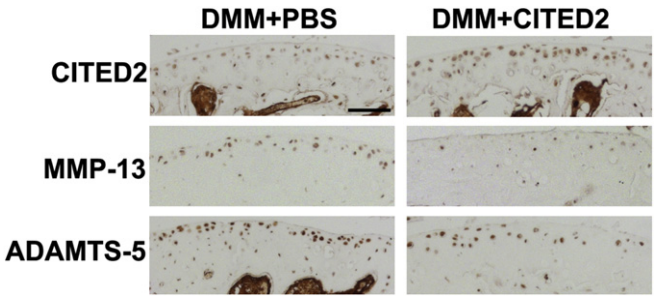
Purpose: Joint injury is a major risk factor for osteoarthritis (OA); up to 50% of patients develop post-traumatic OA after joint injury. Cartilage degradation in OA is mediated primarily by proteolytic enzymes that include members of the MMP (matrix metalloproteinase) and ADAMTS (A Disintegrin And Metalloproteinase with Thrombospondin motifs) families. There are still no effective treatments to prevent or slow progressive joint tissue degradation; however, studies in our laboratory suggest that the transcriptional regulator CITED2, which suppresses expression of multiple MMPs (-1, -3, -13), plays a chondroprotective role. In humans and mice, CITED2 expression is lower in OA cartilage than in healthy controls, suggesting that reduction in CITED2 may contribute to OA pathogenesis. Suppression of CITED2 by introducing CITED2 shRNA into joints also upregulated MMP-13 and ADAMTS-5 and led to OA-like histologic changes. In contrast, gene transfer of CITED2 into joints of rats with inflammatory arthritis limited cartilage matrix destruction. Therefore, in this study we tested whether CITED2 gene transfer would similarly prevent OA onset in a mouse model of post-traumatic OA due to meniscal injury.
Methods: CITED2 gene transfer in vivo and OA induction: Male C57BL/6 mice (8-10-wk old) received intra-articular injections of plasmids encoding CITED2 cDNA (10 mg/10 μL PBS) in the right knee, followed by electroporation (225V, 100ms pulse length, 4 pulses each polarity) every 7 days; left (control) knees received 10 μL PBS followed by electroporation (n=6). To induce osteoarthritis, destabilization of the medial meniscus (DMM) was then carried out by transecting the medial meniscotibial ligament (MMTL) of both limbs. Tissue analyses: Samples from each knee joint were prepared for histology and for RNA isolation. Formalin-fixed, decalcified sections were stained overnight at 4°C with anti-CITED2, anti-MMP-13, anti-ADAMTS-5 or irrelevant isotype-matched antibody controls, followed by anti-rabbit or anti-mouse secondary antibody and visualized with DAB chromagen. Safranin O-Fast green staining was used to detect proteoglycans. Expression of CITED2, MMP-13, and ADAMTS5 in articular cartilage was assessed by SYBR green real-time qPCR.
Results: Four weeks after DMM, joints receiving CITED2 by gene transfer (DMM+CITED2) showed elevated mRNA levels of CITED2 and reduced mRNA levels of MMP-13 and ADAMTS5 compared to DMM joints receiving PBS (DMM+PBS) (Fig 1). Histologically, DMM+CITED2 mice exhibited reduced cartilage degradation (OARSI score 0.33 ± 0.28) compared to DMM+PBS controls (OARSI score: 1.33 ± 0.28) (Fig 2). Immunohistochemistry showed that DMM+CITED2 mice had an increased number of chondrocytes expressing CITED2 and fewer chondrocytes expressing MMP-13 and ADAMTS-5 than DMM+PBS controls (Fig 3).
Conclusions: Our previous studies suggested that reduced CITED2 expression in cartilage contributes to OA pathogenesis. Our present results support the hypothesis that restoration of CITED2 in OA joints by gene transfer would limit disease progression and specifically implicate MMP-13 and ADAMTS5 as targets for CITED2 downregulation. This suggests that increasing CITED2 expression in articular cartilage may be a novel chondroprotective strategy for the treatment of OA.







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EFFECTS OF SINGLE RAAV-MEDIATED TGF-BETA OVEREXPRESSION OVER TIME IN HUMAN OSTEOARTHRITIC ARTICULAR CARTILAGE

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Purpose: rAAV vectors are promising tools to directly apply candidate genes in osteoarthritic (OA) cartilage. Here, we analyzed the effects of TGF-β overexpression via rAAV upon the structure of human OA cartilage *in situ* using one single application over a prolonged period of time.

Methods: The vectors were packaged, purified, and titrated using standard protocols. Human normal articular cartilage was obtained from unaffected areas in knee joints removed during tumor surgery (n = 10) and human OA cartilage from joints undergoing total knee arthroplasty (n = 12) (Mankin score 7-9). Explants and chondrocytes were prepared using standard protocols. Cells and explants were transduced with 40 μl vectors for up to 30 days. The DNA contents were assayed using Hoechst 33258, the proteoglycan contents by binding to DMMB, and the type-II and type-X collagen contents by ELISA. Paraffin-embedded sections were stained with safranin O/H&E and to detect TGF-β, type-II and type-X collagen. TGF-β was also monitored by ELISA. Proliferation was evaluated by immunolabeling following BrdU incorporation. Morphometric measurements were performed at 3 standardized sites along the explant surface by image analysis. Each condition was performed in duplicate in 3 independent experiments. The t-test and Mann-Whitney Rank Sum Test were employed with $P \leq 0.05$ considered statistically significant.

Results: TGF-β production was sustained in rAAV-hTGF-β chondrocytes compared with rAAV-*lacZ* (184.2 *vs.*11.3 pg/ml/24 h in normal cells, 219.4 *vs.* 10.6 pg/ml/24 h in OA cells, up to 21-fold difference, always $P \leq 0.001$). The % of cells positive for BrdU uptake (Fig. 1) and the contents of DNA, proteoglycans, and type-II collagen were always higher in the rAAV-hTGF-β normal and OA cells compared with rAAV-*lacZ* (up to 12-fold, always $P \leq 0.001$). The type-X collagen contents significantly decreased in OA cells with rAAV-hTGF-β (1.6-fold, $P \leq 0.001$). TGF-β production was also sustained in rAAV-hTGF-β explants compared with rAAV-*lacZ* (724.5 *vs*. 92.3 pg/ml/24 h in normal cartilage, 987.7 *vs*. 83.4 pg/ml/24 h in OA cartilage, up to 12-fold difference, always $P \leq 0.001$) (Fig. 2). The cell densities (Fig. 3), % of cells positive for BrdU uptake (Fig. 4), DNA contents, proteoglycan contents and matrix staining intensity (Fig. 3), and type-II contents and immunoreactivity (insets of Fig. 3) were always higher in the rAAV-hTGF-β normal and OA explants compared with rAAV-*lacZ* (up to 23-fold, always $P \leq 0.001$). The type-X collagen contents and immunoreactivity significantly decreased in OA cartilage with rAAV-hTGF-β (4-fold, $P \leq 0.001$) (Fig. 5).

Conclusions: TGF-β can be overexpressed in human normal and OA chondrocytes via rAAV *in vitro* and *in situ*, leading to enhanced cell proliferation and matrix synthesis and to OA cartilage remodelling with reduced hypertrophic type-X collagen expression. Administration of the vector *in vivo*, probably combined with other favorable candidates, will allow to determine the beneficial effects of the approach on reconstructing OA cartilage to avoid osteophyte formation.

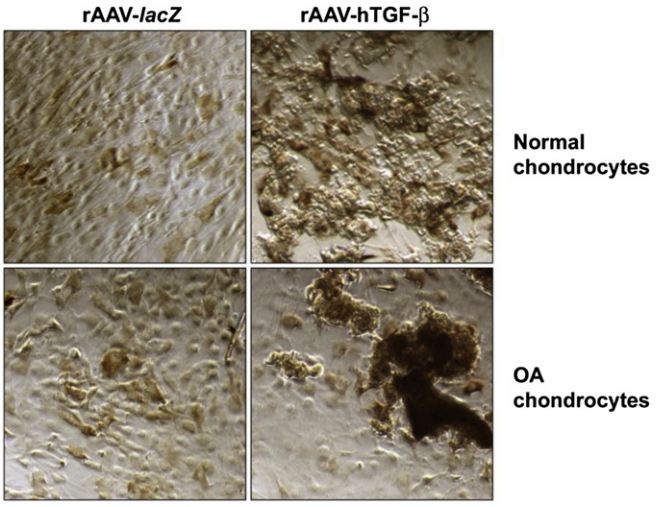

Fig. 1. Immunocytochemical detection of BrdU in transduced human normal and OA chondrocytes (day 30). Magnification x10.

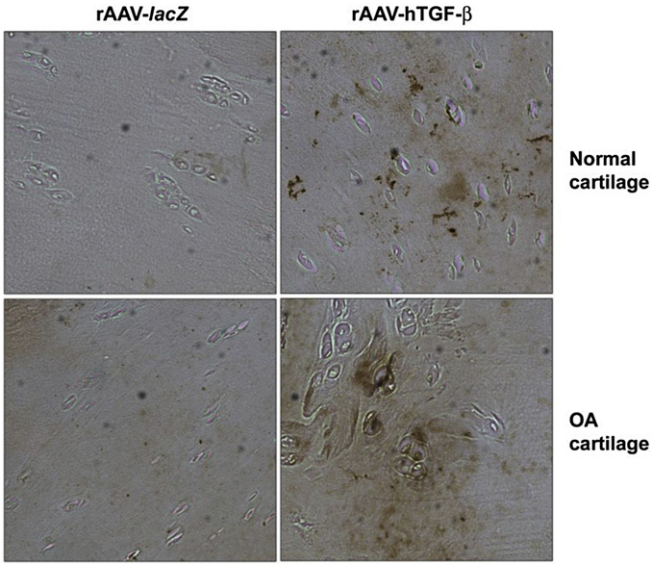

Fig. 2. Immunohistochemical detection of TGF-β in transduced human normal and OA cartilage (day 30). Magnification x20.